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Quality of scaffold fixation in a human cadaver knee model

J. E. J. Bekkers†, A. I. Tsuchida†, J. Malda†, L. B. Creemers†, R. J. M. Castelein†, D. B. F. Saris†* and W. J. A. Dhert†‡

† *Department of Orthopaedics, University Medical Center Utrecht, 3584CX Utrecht, The Netherlands*

‡ *Faculty of Veterinary Medicine, University of Utrecht, 3584CX Utrecht, The Netherlands*

Summary

Objective: Newly developed regenerative cartilage interventions based on the application of 3D-scaffolds require a further evaluation of the surgical techniques involved. The present study compared four different scaffold fixation techniques [fibrin glue (FG), transosseous (TS) fixation, biodegradable pin (BP) fixation and continuous cartilage sutures (CS)] to implant a custom-printed porous PEOT/PBT1000/70/30 scaffold in a human cadaver knee model.

Methods: After implantation, the knees were subjected to a vertically oriented loaded continuous passive motion (CPM) protocol. The fixation techniques were evaluated after 60 and a subsequent 150 motion cycles, focusing on area coverage, outline attachment and scaffold integrity. After the total of 210 cycles, also an endpoint fixation test was performed.

Results: The fixation techniques revealed marginal differences for area coverage and outline attachment after 60 and 150 cycles. The FG scored higher on scaffold integrity compared to TS ($P < 0.05$) and CS ($P = 0.01$). Endpoint fixation was highest for the CS, whereas FG showed a weak final fixation strength ($P = 0.01$).

Conclusions: This study showed that optimal fixation cannot be combined always with high scaffold integrity. Special attention devoted to scaffold properties in relation to the fixation technique may result in an improvement of scaffold fixation, and thus clinical cartilage regenerative approaches involving these scaffolds.

Key words: Scaffold, Fixation, PEOT/PBT, Cartilage regeneration, Focal cartilage lesion.

Introduction

Autologous Chondrocyte Implantation (ACI) has proved to be a successful treatment for focal cartilage lesions. Ongoing improvements of this regenerative cartilage therapy have lead to the implementation of 3D resorbable scaffolds[1]. Several different biomaterials, including collagen type I/III bilayer matrices, Hyalograft® C and BioCart™II[2–4] have already been applied as scaffolds in humans with promising results.

Although good results have been published on the use of natural materials to enhance cartilage formation both *in vitro*[5] and *in vivo*[6–8], synthetic materials are in favor because of their limited risk on pathogen transfer, lower batch-to-batch variation and their ability to be mass produced[9,10]. The most frequently investigated synthetic biomaterials used for cartilage regenerative purposes includes polyglycolic acid (PGA), polylactic acid (PLA), poly (lactic-co-glycolic) acid (PLGA) and poly (ethylene glycol)-terephthalate/poly (butylene terephthalate) (PEOT/PBT)[10,11]. The PEOT/PBT copolymer showed an enhanced chondrocyte redifferentiation and cartilage matrix formation[12,13]. Varying the amount and the length of the hydrophilic PEOT and hydrophobic PBT block, offers extensive possibilities in the design of polymer systems with tailor-made properties, such as swelling, degradability and mechanical strength[10].

In the development of scaffold-based approaches in cartilage regeneration therapy, the clinical handling and application has been largely overlooked, despite the fact that these are also likely to influence treatment outcome. Therefore, the choice of techniques used for *in situ* fixation of the scaffolds merits attention. Currently, continuous cartilage sutures (CS) are being used for the fixation of collagen membranes in ACI and fibrin glue (FG) is applied as an additional sealant. In addition, some new techniques, such as biodegradable pin (BP) fixation and transosseous (TS) fixation, have recently become available for the fixation of scaffolds[14,15]. To the best of our knowledge, there are only two reports that compare several fixation techniques for scaffold implantation in a knee in a human cadaver *ex vivo* model[16,17].

Therefore, we tested a PEOT/PBT copolymer scaffold, combined with four different scaffold fixation techniques, namely CS, BP fixation, TS sutures and FG, to implant a printed PEOT/PBT 1000/70/30 scaffold in a human cadaver knee model, exposed to a loaded vertically oriented continuous passive motion (CPM) protocol and focused on scaffold attachment and fixation technique-related scaffold damage.

*Address correspondence and reprint requests to: D. B. F. Saris, Orthopaedic Surgeon, Department of Orthopaedics, University Medical Center Utrecht, PO Box 85500, 3508 GA Utrecht, The Netherlands. Tel: 31-88-756971; Fax: 31-30-2510638; E-mail: d.saris@umcutrecht.nl

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Material and methods

POROUS SCAFFOLD FABRICATION

PEOT/PBT copolymer granules were obtained from PoroGen B.V. (Bilthoven, The Netherlands) with a composition denoted as 1000/70/30, where 1000 represents the PEG molecular weight (MW) (g/mol), 70 and 30 represent the wt% of the PEOT and PBT blocks, respectively. Porous scaffolds were produced using a 3D fiber deposition technique[18] using the BioScaffolder system (SysEng, Hünxe, Germany). Copolymer granules were placed in the dispense head and allowed to melt at a temperature of 200°C. Molten PEOT/PBT copolymer was forced through a 27 gauge needle (DL Technology, Haverhill, MA) by pressure. The printing of pre-designed scaffolds was controlled by a deposition program (PrimCam version 3.0) via the printer port. Using a printing speed of 262.5 mm/s, a spindle speed of 200 rpm and a fiber spacing of 600 μm, fibers were successively laid down on a heated deposition platform (40°C) in a 0°–90° orientation, creating a consistent pore size and 100% interconnecting pore volume (Fig. 1). Scaffolds printed according to these settings were previously shown to typically have a porosity of approximately 60% and a dynamic stiffness of 1 MPa[18]. Scaffolds were printed either 7 or 14 layers thick, in order to be flush with the created defect. To allow for swelling of the scaffolds, they were immersed in phosphate-buffered saline overnight. After overnight immersion the 7 and 14 layered scaffolds reached a thickness of 1 and 2 mm, respectively. The prepared scaffolds were cut out to match exactly the debrided lesions' geometry, using the same template used to create the cartilage defect.

CREATION OF THE FULL-THICKNESS CARTILAGE DEFECT

Twenty human cadaver legs were obtained from six male and six female donors (age range: 53–91 years) in accordance with the guidelines of the local ethical committee. Prior to inclusion in the study, the extremities were tested for malalignment, knee instability and full extension and 80° flexion of the knee. The knees were opened using a medial parapatellar approach with lateralization of the patella to obtain a full view of the articular cartilage of the central regions of the femur. Both medial and lateral femur condyles were used for scaffold implantation. A custom-made template with a surface area of 2 cm^2 was used to demarcate the outline of the cartilage defect at the load-bearing portion of each medial and lateral femur condyle. Following this a sharp surgical spoon was used to debride the full-thickness articular cartilage defects. After implantation of the PEOT/PBT scaffolds, the intraarticular environment was filled with phosphate-buffered saline, to allow for lubrication during the CPM protocol, and the knees were closed in layers.

FIXATION TECHNIQUES

The four fixation techniques applied in this study, FG (Tissuecol®, Baxter), modified TS fixation[14], BP fixation[15] (SmartNail®, ConMed Linvatec) and continuous CS (Vicryl®6.0, Ethicon), were randomly assigned to either the medial or lateral femoral condyle of the 20 cadaver knees, thus 10 implantations per fixation technique (Fig. 2).

FG (Tissuecol)

The FG was kept in hot water (approximately 37°C) prior to application, following the manufacturer's instructions. The created cartilage defect was dried and the warmed FG was applied at the bottom of the defect and towards the edges of the cartilage rim. Following this, the scaffold was placed into the defect and additional FG was added at the cartilage-scaffold interface. Before the knee was closed the FG fixation was allowed to dry under a continuous hot air flow for 10 min with continuous rinsing of the surrounding articular cartilage surface to keep it sufficiently moist.

CS

The scaffold was placed into the defect and sutured to the adjacent cartilage rim by continuous Vicryl® 6.0 bioresorbable sutures comparable to the, previously described, fixation procedure of the ACI technique[19].

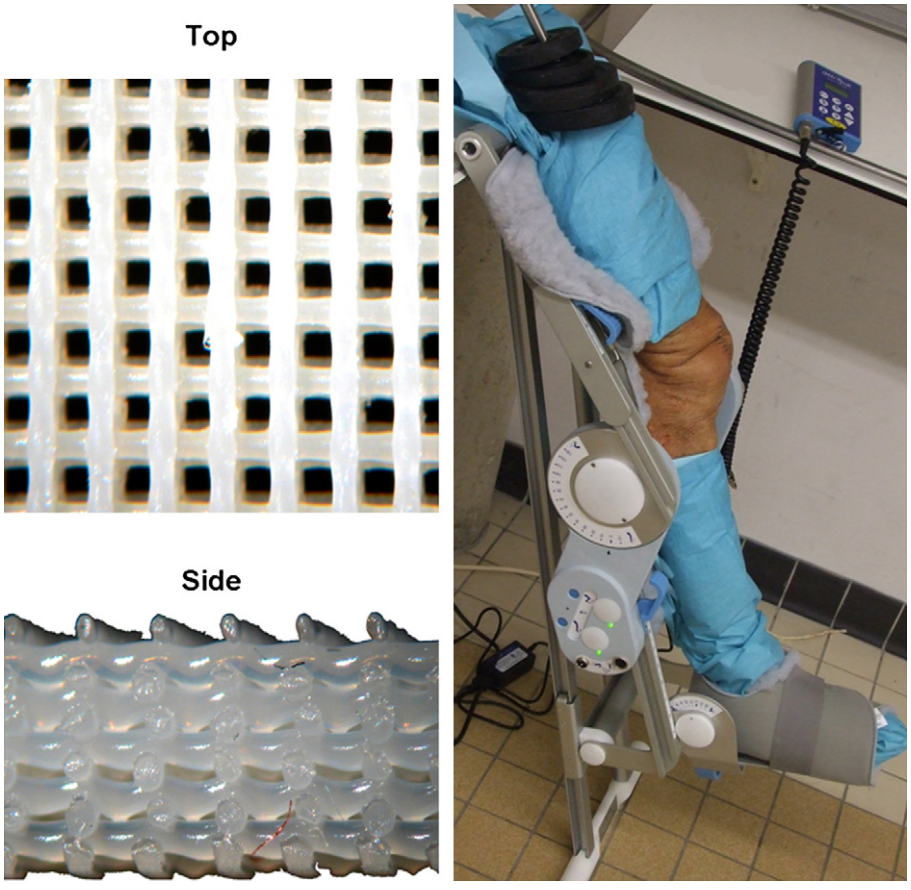

Fig. 1. The PEOT/PBT polymer scaffold and the applied test setup.

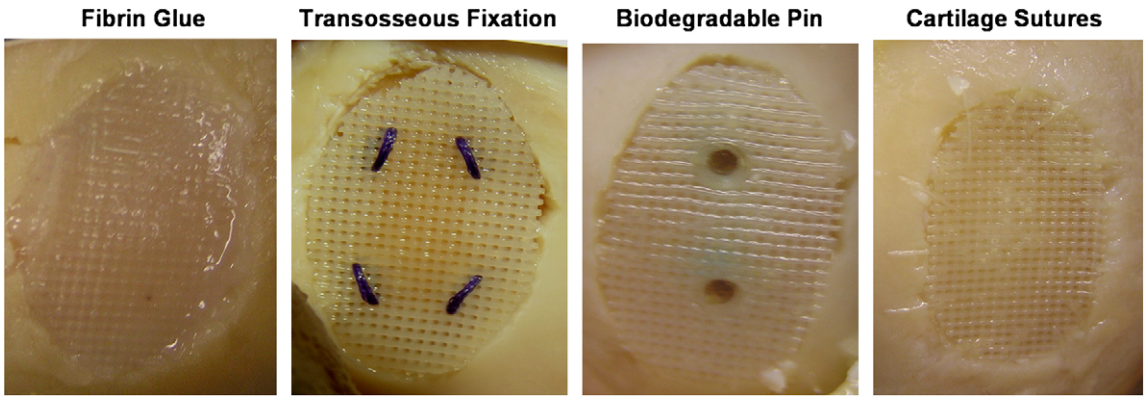

Fig. 2. Scaffold fixation techniques.

BP (SmartNail®)

For this technique a 1.5 mm K-wire was used to drill two 14 mm deep holes into the subchondral bone at 1/3rd and 2/3rd height of the defect. Following this the scaffold was placed into the defect and the SmartNails® were tapped, through the scaffold, into the drilled wholes to provide a firm fixation of the scaffold into the created cartilage defect.

TS sutures

A guide wire (1 mm diameter) was used to drill four holes at the 2, 4, 8 and 10 o'clock locations of our defect. At the same locations the scaffold was armed with a resorbable 2.0 thread. At respectively 1 cm and 2 cm from the scaffold, 3- and 2-fold knots were created. The threads were pulled into the defect and through the femoral bone by the guide wires. At the end a short, firm action was necessary to pull the knots into the drilled holes to anchor the scaffold into the cartilage defect.

CPM PROTOCOL

After implantation of the scaffolds, the legs were strapped onto the CPM devices (Össur, Son en Breugel, The Netherlands and Firma Medical SOT B.V., Losser, The Netherlands) oriented in a vertical position (Fig. 1). A total load of 35 N was attached on top of each leg to allow for a continuous axial force during motion. One motion cycle was defined as full extension (0°) to 80° flexion to again full extension (0°) and lasted for approximately 60 s. After 60 loaded continuous cycles, the knees were reopened and the implantation sites photographed for later macroscopic analysis. Knees were then closed again and exposed to an additional consecutive 150 loaded continuous motion cycles. Subsequently, the knees were opened again and photographs were taken.

ANALYSIS OF FIXATION

Scoring system

After 60 and after a consecutive 150 cycles, the obtained pictures from the implantation sites were evaluated by two independent observers using a modified scoring system[16] focusing on area coverage, outline attachment and scaffold integrity (Table I). Area coverage and outline attachment were calculated using the AnalySIS 3.0 software. The length of the outer cartilage defect rim was marked to calculate the defect surface area and outline, which were correlated to the scaffold surface area and outline, presented as percentages. Scaffold integrity was determined, on a 5-point scale, by evaluating the severity and location of scaffold fissures and cracks with concomitant focal or generalized scaffold disorganization (Table I). After the total of 210 cycles, an additional endpoint fixation test was performed. A suture was pulled through the center of the scaffold and connected to a pulley-block system to quantitatively measure the pull-off force necessary to dislocate the scaffold.

Scaffold damage

After the loaded CPM test, scaffolds were evaluated macroscopically using a stereomicroscope (Zeiss, Stemi-2000C, Germany) with a 10× magnification. Detailed longitudinal and transverse images of the scaffolds were obtained to show the fixation technique-related scaffold damage.

Statistical analysis

For each individual fixation technique, the average scores and standard deviations per scoring item were calculated after both 60 and 150 motion cycles. Differences in quality of the scaffold fixation techniques, per scoring item, were analyzed for statistical significance by a non-parametric Kruskal–Wallis test followed by a multiple comparison test and Bonferroni correction.

Table I
Modified scoring system

Outline attachment*	Area coverage†	Scaffold integrity	Endpoint fixation
Unchanged (5)	Unchanged (100%) (5)	Unchanged (5)	Cannot be detached/ suture is pulled through the scaffold. (5)
<25% (4)	75–100% (4)	Shape deformities or minor fissures that are unrelated to fixation (4)	Detached with 2.5–3 N (4)
25–50% (3)	50–75% (3)	Minor fissures or cracks close to the fixationsite (3)	Detached with 1.5–2 N (3)
50–75% (2)	25–50% (2)	Fissures or cracks that jeopardize the fixation of the scaffold (2)	Detached with 0.5–1 N (2)
75–100% (1)	<25% (1)	Fissures or cracks jeopardizing the fixation with surrounding scaffold disorganization (1)	Detached with ≤0.5 N (1)
100% (0)	0% (0)	Fissures or cracks jeopardizing the fixation with generalized scaffold disorganization (0)	Total self detachment (0)

*% of full circumference that has lost contact with the surrounding cartilage rim.
†% of total cartilage defect that is covered by scaffold.

Table II
Results after 60 and 150 cycles

	FG	TS	BP	CS
60 cycles average score (±standard deviation)				
Outline attachment	2.7 (±1.89)	1.9 (±1.10)	1.9 (±1.10)	3.2 (±0.92)
Area coverage	2.8 (±1.93)	3.9 (± 1.26)	3.2 (±1.69)	4.1 (±0.32)
Integrity	**4.3 (0.48)***	**1.9 (1.04)***	2.3 (±1.34)	2.3 (±1.51)
150 cycles average score (±standard deviation)				
Outline attachment	2.4 (±1.78)	1.8 (±1.03)	1.6 (±0.97)	3.0 (±0.82)
Area coverage	**2.8 (1.93)#**	3.6 (±1.26)	3.2 (±1.69)	**4.0 (0.00)#**
Integrity	**4.1 (0.63)+**	1.7 (±0.95)	2.3 (±1.34)	**1.2 (1.13)+**

Average per scoring item for the scaffold fixation techniques after 60 and 150 cycles. (*$P < 0.05$, #$P = 0.01$, +$P = 0.01$).

Results

PROCEDURES

Chondral defects were only present in four legs and these defects could easily be debrided during the creation of the full-thickness cartilage defect. All fixation techniques were straightforward, although fixing the scaffolds using TS or continuous CS was more time consuming compared to the fixation using BP or FG. Evaluation after 60 and the additional 150 cycles of loaded CPM revealed no significant difference between medially and laterally implanted scaffolds and between 7 and 14 layered scaffolds for any of the scoring items (data not shown), therefore subsequent analyses of the scaffold fixation techniques was done regardless of scaffold thickness or implantation site. None of the specimens showed macroscopic damage at the opposing articular cartilage surface (data not shown).

AREA COVERAGE

With regard to area coverage, marginal differences were observed between the four different fixation techniques after both 60 and subsequent 150 cycles of loaded CPM (Table II). After 60 cycles, 3 out of 10 scaffolds fixed with FG, 2 out of 10 scaffolds fixed with BPs and 1 out of 10 scaffolds fixed with TS sutures, were completely detached. The remaining scaffolds did not detach after the additional 150 cycles. Closer examination of the implantation sites revealed either nearly complete attachment or total detachment of the scaffold, accounting for a large variation in obtained area coverage score for these three techniques (Table II). If total detachment occurred during the motion cycles, full-thickness scaffold fissures were observed at the fixation sites for the BP and TS fixation techniques (Fig. 3), whereas scaffolds fixed by continuous CS remained stable after 60 and subsequent 150 cycles. However, an occasional rupture of a scaffold fiber, caused by the articular CS, was observed resulting in loss of scaffold material [Fig. 3(B)] and thus lower area coverage.

OUTLINE ATTACHMENT

For the BP, and to a lesser extent the TS suture fixation, folding at the edges was regularly noticed. Small differences were present between the fixation techniques in outline attachment (Table II). Lower scores, however not statistically significant, were observed for the BP and TS sutures due to folding of the scaffold and suture rupture respectively. In addition, for the TS suture technique, a minor discrepancy was noticed between the drilled holes and the site of suture fixation in the scaffold, allowing for dislocation of the scaffold and thus reducing outline attachment. The outline attachment score for articular CS was higher compared to the other three techniques, however not significant (Table II).

SCAFFOLD INTEGRITY

The BP, TS suture and continuous cartilage suture fixation had slightly damaged the scaffold during the fixation procedure. This technique-associated damage increased during loaded CPM and led to subsequent lower scaffold integrity scores (Fig. 3). For CS, as well as for the BP and TS sutures, full-thickness scaffold fissures and cracks were noticed after 60 cycles. Moreover, after 150 cycles, generalized scaffold disorganization was regularly noticed when articular CS were applied (Fig. 3). The FG fixation technique provided the best scaffold integrity as compared to TS sutures after 60 ($P = 0.04$) and 150 ($P = 0.07$) cycles and CS ($P = 0.01$) after 150 cycles.

ENDPOINT FIXATION

The endpoint fixation test showed a statistically significant better final scaffold attachment ($P = 0.01$) for the continuous cartilage suture compared to the FG fixation techniques (Fig. 4).

Discussion

Secure delivery and retention of a cartilage tissue engineered construct is important for a successful outcome after regenerative cartilage therapy. However, the clinical handling and application of these constructs has been largely overlooked. In the present study, four different scaffold fixation techniques for the implantation of a printed PEOT/PBT scaffold in human cadaver knee joints were compared. We focused on the effect of loaded motion on attachment of the scaffold into the debrided defect and on scaffold damage.

FG provided an excellent protection of the scaffold integrity during loaded motion and, if not detached, a good area coverage. The BP, TS and cartilage suture fixation techniques caused minor damage to the scaffolds during application, leading to further deterioration of the scaffold composition during loaded motion. Elastic properties of the implanted scaffold will determine folding and or dislocation at the outer margins of the defect when a two or four-point fixation technique is applied, since these techniques do not provide any fixation at the outer margins[16].

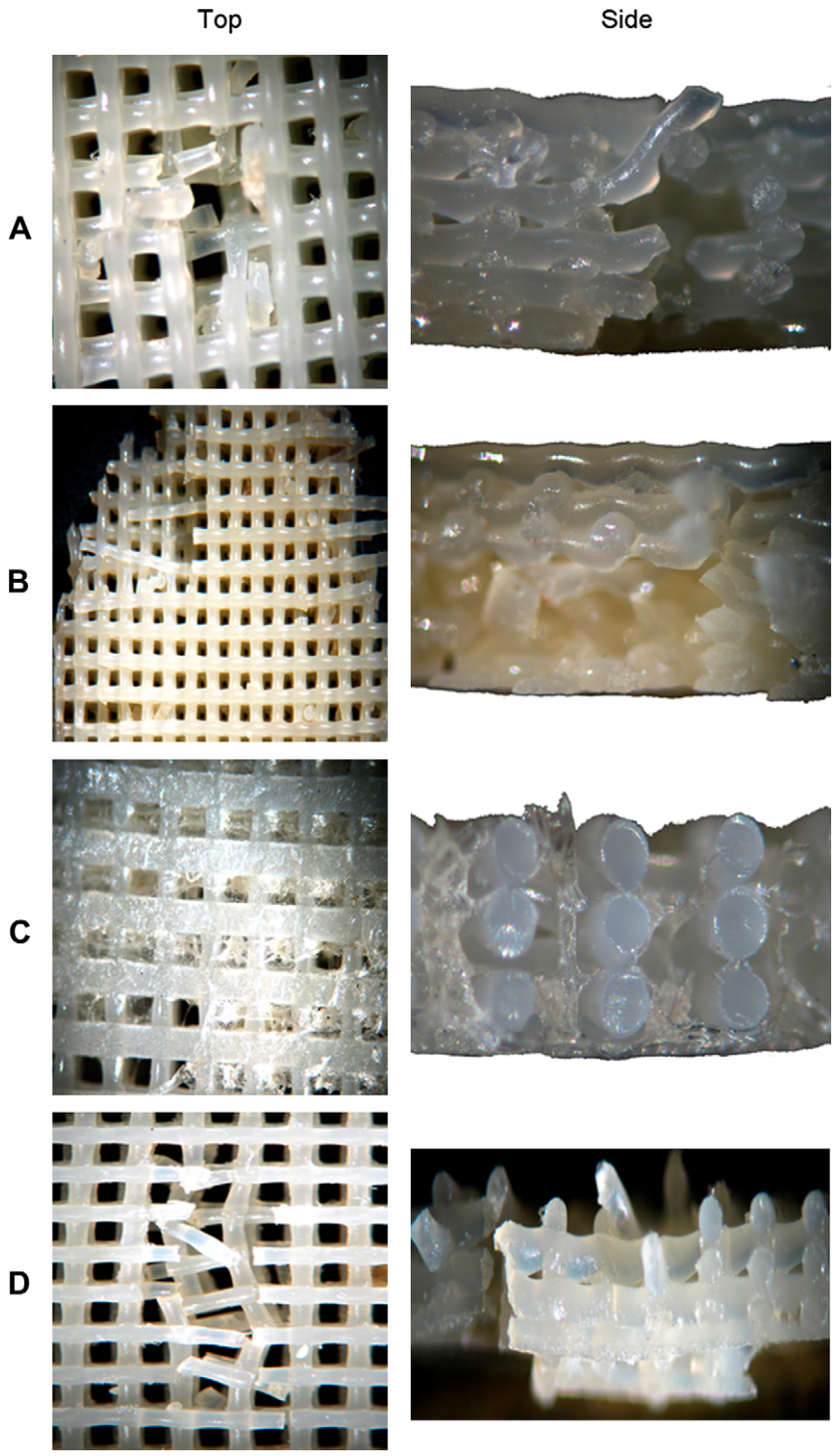

Fig. 3. Confocal stereomicroscopic pictures of the scaffolds after fixation. Representative stereomicroscopic pictures of scaffolds after 150 CPM cycles for the applied fixation techniques (BP (A), CS (B), FG (C) and TS sutures (D)) showing a disorganized collapsed scaffold composition with full-thickness fissures or partial scaffold loss for techniques A, B and D.

Techniques where fixation occurs at the outer margins of the defect, like CS and FG, do provide good outline attachment. In our study, however, FG provided a weak attachment when compared to the suturing techniques. This is both remarkable and worrisome, especially when considering its broad clinical application. One explanation for this finding might be that FG is a biological sealant and therefore the *ex vivo* performance could be inferior, e.g., lacking the additional fixation by the blood normally present during knee surgery or the lubricating properties of the synovial fluid. In addition, the scaffold used in the current study, which is printed and rather stiff, could be less suitable to be combined with FG compared to the ones currently being used in clinical practice.

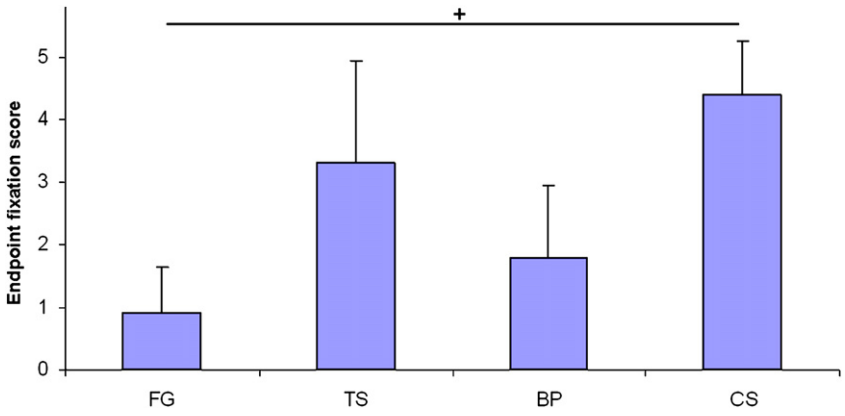

Fig. 4. Endpoint fixation. Endpoint fixation score per fixation technique ($+P = 0.01$).

During the endpoint fixation test, the pulley-block system applies a force perpendicular to the scaffold surface, which is different from the intraarticular shear stresses created during walking. Although the effect of the cartilage defect rim on scaffold stability is unclear we believe that the pulley-block system is a good measure of the actual attachment of the scaffold into the created cartilage defect.

Our findings are in line with previously reported work on scaffold fixation techniques by Knecht *et al.* Different fixation techniques and scaffold types were compared by uniaxial tensile tests showing that, regardless of the type of scaffold material used, suturing techniques provide better attachment of the scaffold to the cartilage defect when compared to FG [17]. This actually would suggest that the scaffold material does not influence fixation quality. However, their study-design, applying non-weight bearing, static uniaxial tensile tests on *in situ* cadaver legs, makes the translation of the results to a clinical setting difficult. Therefore, the real behavior of a scaffold fixed by a certain technique can better be analyzed in an intraarticular environment. Drobnič and colleagues tested the fixation of a collagen fleece with four different techniques in an intraarticular environment by the use of a CPM protocol in a horizontal orientation[16]. Similar to our observations on the BP and TS fixation, they also noticed deformation of the scaffold when applying their 2-point fixation technique. In addition, FG provided high integrity scores compared to the suturing techniques while the opposite held true for the endpoint fixation. However, the loaded vertically oriented CPM test model of the current study is likely to be a better *ex vivo* approximation of the intraarticular influences on the implanted construct during the postoperative rehabilitation program after cell-based cartilage therapy. Although we did not perform intraarticular pressure measurements, it is likely that the vertical orientation of the cadaver legs with additional loading during motion generated a shear force on the implanted construct, analogous to the *in vivo* situation. This shear force will partially be mediated directly by friction with the opposing cartilage and partially by intraarticular fluid flow. Further research is needed to demonstrate whether perhaps a slightly countersunk scaffold could protect it from the shear forces *in vivo*, although in one rabbit study it was shown that countersinking metal implants in cartilage defects negatively affected the integrity of the articulating cartilage surfaces[20].

Although the *in vitro* optimization of conditions for cartilage regeneration is important, the eventual *in vivo* circumstances might even be more important. For example, several components of the synovial fluid are believed to inhibit the integration of a cartilage construct into the adjacent tissue[21–23]. In addition, a suboptimal contact between the engineered constructs and the native tissue, e.g., due to folding of the construct at the outline, will negatively influence the integration of the construct with the surrounding tissue. Moreover, failing scaffold fixation can result in a loose intraarticular body, damage to the articulating cartilage surface and loss of reparative cells at the site of the defect. Therefore, stable and lasting *in vivo* fixation with preservation of the 3D-construct geometry is mandatory to profit from the added value of this scaffold-based tissue engineered approach in regenerative cartilage therapy.

The discrepancy between scaffold integrity and endpoint fixation tests in the current study, suggests that good fixation of a scaffold can only be achieved by using techniques that will compromise the scaffold integrity. This raises the question whether the focus for *in vivo* application of 3D-scaffolds should be optimal fixation or scaffold preservation. Adaptation of scaffold architecture, e.g., preprinted holes when applying the BP fixation technique, will most likely limit the scaffold damage as initiated during fixation technique application. This opens the possibility to apply fixation techniques providing a secure fixation while maintaining integrity. Another consideration in the choice of a certain fixation technique may be a reduction of patient morbidity by using a mini-open arthrotomy or an arthroscopic approach. In contrast to the TS sutures, FG and BP fixation, the cartilage suturing technique cannot be applied by either of these approaches and requires an arthrotomy.

The limited number of publications on clinical handling of 3D-scaffolds for *in vivo* application in knee surgery is an indication of the lack of attention in this area. Improvement of the interdisciplinary communication between materials research and surgical technique development has previously been described as a challenge[24] and is mandatory to improve the translation of basic material science towards clinical concepts. The combination of knowledge on scaffolds for cartilage repair and surgical skills and techniques will lead to optimal scaffold fixation with limited damage to the scaffolds.

Conclusion

This study showed that optimal fixation cannot always be combined with high scaffold integrity. Special attention devoted to the effect of the applied fixation technique on the scaffold integrity will result in an excellent scaffold fixation and integrity preservation for future clinical application.

Conflict of interest

The authors declare no conflict of interest related to this manuscript.

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